REMARKS

The specification has been amended to insert a reference to SEQ ID NO:11 when referring to the TACE amino acid sequence disclosed in Table 1, as requested by the Office. A revised Sequence Listing in computer readable and paper form was submitted to the USPTO on October 29, 2007, together with a verification statement to fulfill the requirements of 37 C.F.R. §1.821-1.825. The amendment to the specification and previous Sequence Listing submission contain no new matter in compliance with 37 C.F.R. §1.821(g).

Claims 5, 14-16, 20, 29-31, 32-33 and 36 have been amended. Claims 6, 10, 13, 17 and 23-28 are cancelled; claims 1-4, 7-9, 11-12, 18-19, 21-22 and 34-35 are reiterated. Claims 37-39 have been added. Upon entry of the amendment, claims 1-5, 7-9, 11-12, 14-16, 18-22, and 29-39 will be pending. No new matter has been added.

Claim 5 was amended to depend from claim 1, instead of claim 4. Claim 15 was amended to incorporate the limitation of claim 17, now canceled. Claims 15 and 32 were amended to specify in the alternative the particular mutations of TACE recited in previously presented claim 5. Support for the amendment to claim 20 can be found, *e.g.*, in paragraph 48 of US 2004/0265983. Support for the amendment to claim 29 and new claims 37-39 can be found, *e.g.*, in paragraph 30 and in Table 1. Claims 29 and 37-38 are directed to crystalline forms of a TACE polypeptide "consisting essentially of" the amino acid sequence specified. It is noted for the record that the TACE polypeptide amino acid sequence recited in these claims may contain additional elements that do not materially affect the basic and novel characteristics of the claims (see M.P.E.P 2111.03, citing *In re Hertz*, 537 F.2d 549). The remaining claims were amendment to correct matters of form.

The claim amendments and cancellations made herein are for the purpose of expediting prosecution of the instant application. Applicants do not acquiesce to the rejections made by the Office, and reserve the right to pursue the canceled subject matter in one or more continuing applications.

Applicants note with appreciation the Office's reconsideration and withdrawal of certain rejections raised in the Final Office Action dated February 27, 2007.

Sequence Compliance

The specification was objected to as failing to comply with the sequence requirements. In response, Applicants have amended the specification to insert the appropriate sequence identifier of the TACE polypeptide amino acid sequence disclosed in Table 1. No new matter has been added. Reconsideration of this objection is respectfully requested.

Claim Objections

Claims 29 and 36 were objected to on page 4 of the Office Action because of certain typographical informalities. In response, these claims have been amended to delete the objected phrases, thus obviating the objections made to claims 29 and 36.

Rejections Under 35 U.S.C. §112, Second Paragraph

On pages 4-5 of the Office Action, the Office has rejected claims 4-5 under 35 U.S.C. §112, second paragraph, as allegedly being indefinite. The Office suggested the Applicants to clarify the scope of the intended polypeptides of the composition of claims 4-5.

This rejection has been met by amending claim 5 to depend from claim 1, instead of claim 4. While Applicants do not concede to any of aspect of the Office's stated reasons for rejection, the amendment of claim 5 renders the rejection of claims 4-5 moot.

The rejection of claims 14, 30 and 34 has also been met by the amendments of these claims made herein to change the order of the phrases "TACE polypeptide" and crystal or crystalline forms as proposed by the Office on page 6 of the Office Action. The amended claims now specify that it is the "TACE polypeptide" and/or the "hydroxamate- based binding partner" of the crystal or crystalline form that has the properties, *e.g.*, the structural coordinates, specified.

With respect to the rejection of claim 20 (and dependent claim 21) as allegedly being indefinite, claim 20 has been amended to clarify that the concentration range specified applies to the TACE polypeptide in solution.

While Applicants do not concede to any of aspect of the Office's stated reasons for the rejections under 35 U.S.C. 112, second paragraph, the amendments made herein render these rejections moot. Reconsideration and withdrawal of these rejections are respectfully requested.

Rejection of Claims 1-5, 7-9, 11-12, 14-22 and 29-31 under 35 U.S.C. §112, First Paragraph

New Matter

In paragraph 15 of the Office Action (pages 7-9), the Office has maintained the position that claims 15-16 and 18-21 allegedly introduce new matter in the phrase "wherein the crystallization buffer comprises sodium citrate." According to the Office, the three disclosed species of crystallization buffers recited in the specification fail to provide adequate descriptive support for this phrase.

While Applicants do not concede to any of aspect of the Office's stated reasons for this rejection, the rejection has been met by the present amendment of claim 15 to recite the three species of crystallization buffers containing sodium citrate disclosed in the specification, namely, 0.1M Na Citrate pH 5.4, 20% w/v PEG 4000, and 20% v/v isopropanol (Buffer "D"); 0.1M Na Citrate pH 5.0 and 40% v/v ethanol (Buffer "B"); and 0.1M Na Citrate 8.7, 20% w/v PEG 4000, and 20% v/v isopropanol (Buffer "C") (*see e.g.*, page 4, lines 3-5; page 16, lines 8-11; page 33, line 21 to page 34, line 9 of the specification). Claim 17 has been cancelled as being redundant in view of the claim amendments made herein.

Therefore, in view of the foregoing, Applicants request that the Office reconsider and withdraw the new matter rejection of claims 15-16 and 18-21.

Written Description

In paragraph 16 of the Office Action (pages 9-18), the Office has maintained the position that claims 1-5, 7-9, 11-12, 14-22 and 29-31 allegedly lacking written description. The Office "acknowledges the amendment to claims 1 and 22 to recite space group $P2_1$ and the unit cell dimensions a=61.38 Å, b=126.27 Å, c=81.27 Å, β=107.41°."

However, the Office maintains the position that the specification fails to adequately describe the claimed composition, particularly with respect to the genus of TACE polypeptides that form a crystal having the recited space group and unit cell dimensions. In particular, the Office states that:

> In contrast to claim 1 of Case 4 of the Trilateral Report, the genus of compositions encompasses crystalline TACE polypeptides and hydroxamate-based binding partners with undefined structure. As noted in prior Office actions, the single disclosed representative species of compositions comprising crystalline polypeptides fails to reflect the substantial variation among the species of the genus, particularly with respect to the structures of the TACE polypeptide and the hydroxamate-based binding partner. In this case, the specification discloses only a single representative species of the genus of recited crystalline forms of a TACE polypeptide, i.e., TACE as disclosed in Black et al., "A Metalloproteinase disintigrin that releases tumor-necrosis-factor-.alpha. from cells," Nature 385:729-733 (February 1997), with Ser266 changed to Ala, Asn452 changed to Gln and the sequence Gly-Ser-$(His)_6$ added to the C-terminus, and expressed in CHO cells co-crystallized with N-[D,L-[2-(hydroxyaminocarbonyl)methyl]-4-methyl-pentanoyl)-L-3-(tert-butyl)-glycyl-L-alanine, having monoclinic space group $P2_1$ and the unit cell dimensions a=61.38 Å, b=126.27 Å, c=81.27 Å, β=107.41°. That the specification discloses *only* these representative species appears to be undisputed by applicant.

Applicants respectfully traverse the Office's maintained position that claims 1 and 22 reciting the parameters of a particular crystal in terms of space group, $P2_1$, and the unit cell dimensions, a=61.38 Å, b=126.27 Å, c=81.27 Å, β=107.41°, are not adequately described in the instant application. Claims 1 and 22 specify the ***exact*** structural parameters of the TACE crystal species disclosed in the present application (*see e.g.*, paragraphs 43-50 of US 2004/0265983). Example 2 (paragraphs 100-108) describes experimental data with explanations on how to make the claimed crystals. For example, paragraph 50 of US 2004/0265983 provides that:

> Another aspect of the invention relates to a TACE polypeptide crystal. One such crystal comprises a TNF-α converting enzyme catalytic domain (TCD) polypeptide co-crystallized with an inhibitor. The crystal diffracts to about 2 A and belongs to the monoclinic space group $P2_1$. The crystal's unit cell comprises four crystallographically independent TCD molecules. The TCD molecules are in an asymmetric unit and are not clustered into separate tetrameres, but are integrated into the infinite periodic structure. The crystal has the cell constants: a=61.38 Å, b=126.27 Å, c=81.27 Å, β=107.41°.

It is undisputed that the scope of claims 1 and 22 is commensurate with the TACE crystal species disclosed by the Applicants. It is also undisputed that claims 1 and 22 in their present form impose almost identical structural parameters as those specified by hypothetical claim 1 exemplified in case 4 of the "Trilateral Project WM4 Comparative Studies in New Technologies: Report on Comparative Study on Protein 3-Dimensional (3-D) Structure Related Claims" released in November 2002 ("the Trilateral Report"). The USPTO indicated in the Trilateral Report that hypothetical claim 1 would meet the written description requirement because the crystal structure of the protein is provided in the claim by specifying the cell unit dimension. More specifically, claim 1 in case 4 of the Trilateral Report is directed to a crystalline form of a known protein P, and reads as follows: "A crystalline form of protein P having unit cell dimensions of a=4.0nm, b=7.8nm, and c=11.0nm." At pages 8 and 66 of the report, the hypothetical specification of case 4 is described as including, *inter alia*, that the inventors have newly produced a stable crystalline form of protein P and that the description gives experimental data with explanations of how to make the crystals. The Trilateral Report, at page 67, referring to the claim of case 4, states that "the claim complies with the written description requirement because the ***structure*** of protein P is provided." (emphasis added).

Like the hypothetical claim 1 presented in case 4 of the Trilateral Report, claims 1 and 22 are directed to a crystalline form of a specific known protein (*i.e.*, TACE), which was characterized in the art prior to the filing date in terms of its structure and function. Also similar to the hypothetical claim 1 presented in case 4, instant claim 1 recites the unit cell dimensions of the crystal. The present specification discloses, *inter alia*, that the inventors had newly produced a crystalline form of TACE, provided TACE sequence information, experimental data with explanations on how to make the crystals, and the three-dimensional structure of a crystalline form of the TACE polypeptide (*see* specification at, *e.g.*, page 2, lines 27-29; page 3, lines 15-20; page 31 line 13 to page 36, line 15; and Table 1). Thus, Applicants respectfully submit that for at least the reasons above, the specification amply provides written description for the crystalline form of a TACE polypeptide as presently set forth in claims 1 and 22.

The Office seems to focus the written description rejection on the alleged breadth of the terms "TACE polypeptide" and "hydroxamate-based binding partner" recited in some of the claims dependent from claims 1 and 22. Applicants remind the Office that "a dependent claim shall include every limitation of the claim from which it depends." (M.P.E.P. glossary definition of dependent claim). Thus, claims 2-5, 7-9, 11-12, 14 (which depend from claim 1) and claims 29-31 (which depend from claim 22) shall be construed to include all the limitations of claim 1 and 22, respectively, incorporated by reference into these dependent claims. More specifically, dependent claims 2-5, 7-9, 11-12, 14 and 29-31, are directed to TACE crystals having the ***exact*** structural parameters of the TACE crystal species disclosed in the present application ***and*** further specifying additional features of the TACE polypeptide and/or the binding partner (*e.g.*, the catalytic domain, particular amino acid sequence, hydoxamate-based binding partner, and/or structural coordinates, among others). Thus, combined with the characterization of the crystal structure of the TACE polypeptide in terms of cell unit dimensions recited in claims 1 and 22, these dependent claims provide ample structural and functional features in common associated with the crystal structure of the TACE polypeptide, alone or complexed with a hydroxamate-based binding partner, to show that Applicants were in possession of the claimed genus at the time the present application was filed.

Applicants note that claims 5, 8, 29, 31, 35 and 36-39 are directed to the very particular species of TACE polypeptide and/or hydroxamate-binding partner (*i.e.*, N-{D,L-2-(hydroxyaminocarbonyl)methyl-4-methylpentanoyl}-L-3-amino-2-dimethylbutanoyl-L-alanine, 2-(amino)ethyl amide) exemplified in the instant application. Reconsideration and withdrawal of this rejection as applied to these claims directed to the particular TACE polypeptide and/or hydroxamate-binding partner exemplified are respectfully requested.

Similarly, claims 32-37 provide sufficient structural and functional features in common associated with the crystal structure of the TACE polypeptide in complex with a hydroxamate-based binding partner to satisfy the written description requirement. Independent claim 32 specifies that the composition comprises a crystalline form of TACE having the sequence specified, wherein the crystalline form has the space group

specified. Claims dependent thereon specify the particular structures of the TCDs, the structural coordinates according to Table 1, the unit cell dimensions, and the particular TACE polypeptide sequence and hydroxamate-based ligand used. The genus of TACE polypeptides encompassed by these claims does not have substantial variation, since all must encode a polypeptide having an amino acid sequence encoded by the sequences specified.

The Office repeats several times throughout the written description rejection that the "specification fails to adequately describe the claimed composition, particularly with respect to the genus of TACE polypeptides and hydroxamate-based binding partners that form a crystal having the recited space group and unit cell dimensions." (Office Action, page 13).

In maintaining this rejection, the Office appears to ignore the structural parameters of the TACE crystals imposed by claims 1 and 22 on dependent claims 2-5, 7-9, 11-12, 14 and 29-31, discussed above, *i.e.*, a particular crystal in terms of space group $P2_1$ and the unit cell dimensions a=61.38 Å, b=126.27 Å, c=81.27 Å, β=107.41°. In addition, the TACE polypeptides recited by the claims were known in the art at the time the application was filed. The amino acid sequences of TACE are disclosed in the instant application, *see e.g.*, paragraphs 3 and 23-30 of US 2004/0265983. For example, paragraph 3 of US 2004/0265983 provides the amino acid sequence, domain structure and characterization of TACE as follows:

> The soluble TNFα is released from the membrane-bound precursor by a membrane-anchored proteinase. This proteinase was recently identified as a multidomain metalloproteinase called TNFα.-converting enzyme (TACE). See, Black et al., "A metalloproteinase disintigrin that releases tumor-necrosis factor-i from cells," Nature 385: 729-733 (1997), Moss et al., "Cloning of a disintigrin metalloproteinase that processes precursor tumor-necrosis factor-α," Nature 385: 733-736 (1997). TACE has recently been identified as a zinc endopeptidase consisting of an extracellular region comprising an N-terminal signal peptide, a pro-domain, a 263 residue catalytic domain (TCD) that is preceded by a furin cleavage site (residues 211-214), a disintegrin domain, an EFF-like domain, and a crambin-like domain, an apparent transmembrane helix and the intracellular C-terminal tail. Tumor necrosis factor-α convertin- g enzme (TACE), including a polynucleotide sequence, is described in detail in the published PCT application No. WO 96/41624, herein incorporated in the entirety by reference.

As acknowledged by the Office, claims 4-5 and 32 limit the scope of the term "TACE polypeptide" to encompass the "expression product" of a polynucleotide encoding amino acids 1-477 of TACE of SEQ ID NO:8. However, the Office interprets this phrase to include "any additional amino acid at the N-terminal and/or C-terminal end(s) of amino acids 1-477 of SEQ ID NO:8.' With respect to claim 4, Applicants submit that regardless of any addition to the N-terminal and/or C-terminal end(s) of amino acids 1-477 of SEQ ID NO:8 encompassed by the claims, as alleged by the Office, the resulting crystal must still have the space group $P2_1$ and the unit cell dimensions a=61.38 Å, b=126.27 Å, c=81.27 Å, β=107.41° required by base claims 1 and 22, ***and*** further require the reference sequence specified.

Applicants further note that claim 5, which now depends from claim 1, and newly added claim 39, recite a TACE polypeptide sequence consisting of the particular species of tagged-TACE polypeptide exemplified in the present application, and therefore, should meet the written description requirement as applied by the Office.

Claim 32 requires the crystal to have the crystalline form of monocyclic space group $P2_1$, in addition to the TACE amino acid sequence specified. Claims 29 and 37-38 are directed to TACE crystals having the structural parameters of the base claim, wherein the TACE polypeptide sequence "consists essentially of" the amino acid sequence disclosed in Table 1 (SEQ ID NO:11). As the Office is aware, these claims may contain additional elements that do not materially affect the basic and novel characteristics of the claims (see M.P.E.P 2111.03). Therefore, the dependent claims further limit the scope of claims 1 and 22 by requiring the particular claimed crystals to have the TACE amino acid sequences specified.

Similarly, claims directed to crystals of TACE complexed with a hydroxamate-based partner (*see e.g.*, claims 7-8, 31, 32 and 35) provide sufficient structural guidance to allow the skilled artisan to readily envision the genus of claimed hydroxamate-based binding partners and understand that applicant invented what is claimed. It is noted that claims 8, 31, 35 and 39 specify the precise species of hydroxamate-based partner exemplified in the present application, namely, N-{D,L-2-(hydroxyaminocarbonyl)methyl-4-methylpentanoyl}-L-3-amino-2-dimethylbutanoyl-L-

alanine, 2-(amino)ethyl amide (see specification, *e.g.*, at paragraphs 6, 14, and 42 of US 2004/0265983. Paragraph 42 of US 2004/0265983 specifically incorporates by reference the disclosure of other TACE inhibitors encompassed by the claim. In light of this disclosure, the skilled artisan would have concluded, at the time of filing of the present application, that Applicants were in possession of the necessary common attributes of the members of the claimed genus.

The Office maintained the rejection of claims 15, as amended in the previous response, and claims dependent therefrom for lack of written description, "particularly with respect to the genus of TACE polypeptides, hydroxamate-based binding partners and crystallization conditions." (Office Action, page 16).

This aspect of the rejection has been met, in part, and is traversed, in part. As amended, claim 15 (and claims depending from claim 15) is directed to a method for crystallizing a TACE polypeptide, comprising, *inter alia*, mixing a solution comprising: (i) a TACE polypeptide that is the expression product of a polynucleotide encoding amino acid residues 1-447 of TACE as depicted in SEQ ID NO:8 or the particular modified variant thereof specified by the claim; and (ii) a hydroxamate-based binding partner, with one of three particular crystallization buffers disclosed in the specification; and crystallizing the mixture by drop vapor diffusion to form a crystalline precipitate.

While Applicants do not concede that any of the claims fail to comply with the written description requirement, claim 15 has herein been amended to specify that the crystallization buffer used in the steps of claim 15 is one of the three citrate buffers disclosed in the instant application, namely, Buffer B: 0.1M Na Citrate pH 5.4, 20% w/v PEG 4000, and 20% v/v isopropanol; Buffer C: 0.1M Na Citrate pH 5.0 and 40% v/v ethanol; and Buffer D: 0.1M Na Citrate pH 8.7, 20% w/v PEG 4000, and 20% v/v isopropanol (*see* paragraphs 48 and 101-102 of US 2004/0265983). These three crystallization conditions provide buffer and pH conditions that were used to successfully crystallize the TACE polypeptides complexed to hydroxamate-buffers, as detailed in Example 2 of the application. For example, in paragraph 105 of US 2004/0265983, the specification provides that "[s]mall crystals were obtained…with either buffer B or C. Further refinement of buffer C resulted in buffer D, which allowed for crystals suitable

for X-ray data collection." The specification also points individually to Buffer D, the refined form of buffers B and C, as an example of the crystallization buffer. Thus, at least three different species of sodium citrate-containing buffers were exemplified in the specification, all three of which provided suitable conditions for crystallization.

Applicants respectfully submit that the presently claimed crystallization methods are amply described by the present specification as one of ordinary skill in the art would have followed the teachings of the specification to produce and purify TACE polypeptides that are the expression product of a polynucleotide encoding amino acids 1-447 of SEQ ID NO:8, and combined the purified product with hydroxamate- based inhibitors (*see e.g.*, Example 1 of the specification, paragraphs 91-99). Crystallization conditions are now specified by the claims and are described in Example 2.

Lastly, the Office cites to Mc Pherson *et al.* and several other references to support the allegation that the state of the art for making a protein crystal at the time of the invention was filed was highly unpredictable. A discussion of these references is provided in response to the enablement rejection below.

In sum, the scope of the aforementioned genus encompassed by the claims does not have substantial variation in view of the claims' precise structural parameters specified by the claims. Given the defined scope of the claims, Applicants respectfully submit that the specification provides ample number of species having a common attribute to show that the applicants were in possession of the claimed crystals and methods. In view of the foregoing, Applicants respectfully request that the Examiner reconsider and withdraw the written description rejections under 35 U.S.C. § 112.

Rejection of Claims 1-5, 7-9, 11-12, 14-22 and 29-31 under 35 U.S.C. § 112, First Paragraph

Enablement

In paragraph 17 (pages 18-31) of the Office Action, claims 1-5, 7-9, 11-12, 14-22 and 29-31 were rejected under 35 U.S.C. § 112, first paragraph as allegedly lacking enablement. The Office cites to the factors enumerated by *In re Wands* 858 F.2d 731, 737 (Fed. Cir. 1988) to support the proposition that the present claims are not enabled. In

particular, the Office states that "the specification only discloses only a single working example of such a diffraction-quality crystal and method of making thereof… the specification fails to provide guidance for crystallizing other polypeptides as encompassed by the claims with an expectation of obtaining diffraction quality crystals optionally having the recited space groups and/or unit cell dimensions." (Office Action at pages 23-24).

Each of the grounds raised by the Office in maintaining the position that the claims are not enabled is discussed in more detail below.

Claim breadth in relation to Applicants' disclosure

Applicants traverse the Office's maintained position that claims 1 and 22, reciting the parameters of the particular crystal exemplified in the instant application in terms of space group, $P2_1$, and the unit cell dimensions, a=61.38 Å, b=126.27 Å, c=81.27 Å, β=107.41°, are not adequately enabled by the instant application. Claims 1 and 22 specify the ***exact*** structural parameters of the TACE crystal species disclosed in the present application (*see e.g.*, paragraphs 43-50 of US 2004/0265983). The crystallization conditions and methods disclosed in the specification resulted in crystals having the space group and parameters disclosed in claims 1 and 22. Applicants do not understand the Office's position in rejecting claims 1 and 22 since the Office admits that the specification discloses "only a single working example of such a diffraction-quality crystal and method of making thereof," and that particular single working example is what is specified by these claims.

Applicants also submit that claims 1 and 22 are commensurate in scope with exemplary claim 1 of case 4 of the Trilateral Report, which was deemed by the USPTO to satisfy the enablement requirement. More specifically, the Trilateral Report states that claims to a crystalline form of a polypeptide (*e.g.*, like exemplary claim 1 of case 4) satisfy the enablement requirement, if the specification teaches how to make the claimed crystals and if one skilled in the art could use the claimed crystal without undue experimentation (see the Trilateral Report at page 67 and case 4 of the Trilateral Report at page 66). The instant specification discloses how to make the claimed composition,

e.g., at page 33-34, and one of skill in the art could have used the claimed crystal without undue experimentation.

Claims 2-5, 7-9, 11-12, 14 (which depend from claim 1) and claims 29-31 (which depend from claim 22) further limit the scope of base claims 1 and 22 by specifying additional features of the TACE polypeptide and/or the binding partner (*e.g.*, the catalytic domain, particular amino acid sequence, hydoxamate-based binding partner, and/or structural coordinates, among others). Thus, combined with the characterization of the crystal structure of the TACE polypeptide in terms of space group and cell unit dimensions recited in claims 1 and 22, these dependent claims further narrow the scope of the base claims.

Applicants note that claims 5, 8, 29, 31, 35 and 36-39 are directed to the very particular species of TACE polypeptide and/or hydroxamate-binding partner (*i.e.*, N-{D,L-2-(hydroxyaminocarbonyl)methyl-4-methylpentanoyl}-L-3-amino-2-dimethylbutanoyl-L-alanine, 2-(amino)ethyl amide) exemplified in the instant application.

Similarly, claims 32-37 are commensurate in scope with Applicants' disclosure. Independent claim 32 specifies that the composition comprises a crystalline form of TACE having the sequence specified, wherein the crystalline form has the space group specified. Claims dependent thereon specify the particular structures of the TCDs, the structural coordinates according to Table 1, the unit cell dimensions, and the particular TACE polypeptide sequence and hydroxamate-based ligand used. The genus of TACE polypeptides encompassed by these claims does not have substantial variation, since all must encode a polypeptide having an amino acid sequence encoded by the sequences specified.

The Office has taken the position that crystal claims that specify the TACE polypeptide as the expression product of a polynucleotide encoding residues 1-477 of TACE of SEQ ID NO:8 (*e.g.*, claims 4 and 32) are not enabled since "the polynucleotide of the claim is open to encoding any additional amino acids at the N-terminal and/or C-terminal end(s) of amino acids 1-477 of SEQ ID NO:8." Applicants disagree with the Office's position since regardless of any addition to the N-terminal and/or C-terminal

end(s) of amino acids 1-477 of SEQ ID NO:8, as alleged by the Office, the resulting crystal must still have the space group $P2_1$ and/or the unit cell dimensions a=61.38 Å, b=126.27 Å, c=81.27 Å, β=107.41° required by base claims 1 from which claim 4 depends and claim 22, ***and*** further require the reference sequence specified.

The specification teaches how to make the claimed crystals, for example at Example 2 (paragraphs 100-108 of US 2004/0265983). The amino acid sequence and domain characterization of TACE were known in the art at the time the instant application was filed and are described in the instant application. For example, a detailed characterization of the location of, and interactions between, residues and domains of TACE, and how these correlate with biological activity is provided, *e.g.,* starting in paragraphs 67 through 80 of the instant application. The location of the active site of TACE was also disclosed in the application (see paragraph 71 and FIG. 2a of the application). The structural coordinates of human TACE were identified as set forth in Table 1 of the application.

The specification also describes at least three species of crystallization buffers that resulted in crystals of TACE polypeptides. The exact crystallization conditions for purifying and crystallizing the TACE polypeptide encompassed by claims 1 and 22 are also provided in the specification. For example, at page 34, the specification states that "[s]mall crystals were obtained…with either buffer B or C. Further refinement of buffer C resulted in buffer D, which allowed for crystals suitable for X-ray data collection" (page 34, lines 1-6). The specification also points individually to Buffer D, the refined form of buffers B and C, as an example of the crystallization buffer (*see* page 4, lines 3-5). Thus, at least three different species of sodium citrate-containing buffers were exemplified in the specification, all three of which provided suitable conditions for crystallization.

As to claims directed to methods of crystallizing TACE polypeptides, amended claim 15 (and claims depending from claim 15) are directed to a method for crystallizing a TACE polypeptide, comprising, *inter alia*, mixing a solution comprising: (i) a TACE polypeptide that is the expression product of a polynucleotide encoding amino acid residues 1-447 of TACE as depicted in SEQ ID NO:8 or the particular modified variant

thereof specified by the claim; and (ii) a hydroxymate-based binding partner, with one of three particular crystallization buffers. Applicants note that the method claims 15-16 and 18-21 are directed to crystallization methods without specifying the crystals to have a particular quality of crystal (e.g., usable for X-ray crystallography). Claim 15, as amended herein, requires the use of one of three crystallization buffers at particular pH and solvent composition. All three buffers now recited by the method claims provided suitable conditions for TACE crystallization, as described above. Accordingly, the breadth of the claims, as amended or newly added herein, is commensurate in scope with the teachings in the specification.

State of the Art / Level of predictability in the art

With respect to the state-of-the-art in generating TACE protein variants, techniques for generating mutant TACE proteins were known in the art and were performed routinely by molecular biologists at the time the present application was filed. The disclosure also describes and demonstrates methods for successfully crystallizing a TACE polypeptide using not one, but three crystallization buffers that comprise sodium citrate *(see* Example 2, paragraphs 100-108). Once the crystallization conditions are established, one of ordinary skill in the art could have practiced the claimed invention, which, as discussed above, is directed to the very specific TACE crystals generated following the conditions outlined in Example 2, paragraphs 100-108, by routine experimentation by following the teachings provided in the specification. Therefore, Applicants submit that following the teachings of the specification, one of ordinary skill in the art would have been able to generate crystals of TACE polypeptide having the structural information encompassed by the claims by simply following the teachings of the specification.

Applicants previously cited Itoh, S. I. and M. A. Navia (1995) *Protein Science,* (4), 2261-2268 and Sauer, U. H., S. Dao-Pin, and B. W. Matthews (1992) *Journal of Biological Chemistry* (267) 2393-2399 as supporting the assertion that at the time the instant application was filed, it was known in the art that variants of proteins with known crystallization parameters were likely to readily crystallize with similar crystal structures

as long as the variations introduced did not markedly affect intermolecular crystal contacts or amino acid residues important for protein stability (*i.e.*, within the hydrophobic core). Even mutations that had an effect in altering protein stability, such as inserting a proline amino acid, were found to crystallize with similar crystallization parameters as the native protein, emphasizing that well-folded proteins can exhibit crystallization properties similar to the non-mutated counterparts. Sauer, U. H., *supra*. The Office dismisses these reference by pointing out that the variants disclosed in the Itoh and Sauer references, particularly in Table 2 in Itoh *et al.* and Table III in Sauer *et a*l. do not maintain the "identical unit cell dimension of the wild-type polypeptide to the extent required by claims 1, 22 and 36, particularly with respect to the dimensions of vectors a, b and c."

The Office's position with respect to the Itoh and Sauer references is traversed. Table 2 in Itoh *et al.* compares the differences between crystals of wild type and two mutants of FKBP12-FK506 complexes. All three FKBP12-FK506 crystals had the same unit cell dimensions of $P4_22_12$, but the a, c values were 58.39 Å, 55.76 Å (wild type), 58.31 Å, 55.93 Å (for a mutant having a conservative substitution of R42K), and 58.25 Å, 55.98 Å (for a mutant having a non-conservative substitution R42I). The Sauer reference showed similar findings in Table III (*i.e.*, 60.9, 96.8 Å (wild type) compared to 60.8, 96.6 Å in a D72P mutant). These references show that the particular mutants disclosed retain the same space group as the parental with type, but have slight differences in unit cell dimensions. Compared to the scope of the present claims, this would indicate that mutants showing a similar extent of variation from the reference TACE crystal structure would be encompassed by claims specifying the space group, but not requiring a specific unit cell dimension (*e.g.*, a claim similar in scope to claim 32), assuming that other conditions are met. The fact that crystals of mutants disclosed by Itoh *et al* and Sauer *et al.* have slight variations in specific unit cell dimensions, while crystallizing in the same space group, from the wild type ***actually supports*** Applicants' arguments that variants of proteins with known crystallization parameters were likely to readily crystallize with similar crystal structures. The Office's statement on page 27 of

the Office Action that these references "support the examiner's position regarding the high level of unpredictability" in the crystallography art is therefore traversed.

The Office Action cites to Ingram *et al.* (2006) *Prot. Eng. Design Select.* 19:155-161 as providing evidence that "structurally similar polypeptides do not form crystals with similar crystal structures." Applicants disagree with the Office's position with respect to Ingram *et al.* It is acknowledged that this reference discloses a mutation of TACE (V353G) that causes a conformational change in the protein that stabilizes it from autoproteolysis. However, the Ingram reference remarks about the conformational change in TACE induced by this mutation and how it "causes a conformational change that facilitates crystallization in a new space group (compared to wild type) that is amenable to soaking." *Id.* at page 161. The mutation at position 353 of TACE disclosed by Ingram *et al.* had a dramatic effect on enzyme activity by stabilizing autodegradation that is also reflected in the protein conformation. The fact that Ingram *et al.* discuss how the change in conformation leads to a new space group indicates that this is one specific example of a significant conformational change. As discussed above in the context of the Itoh and Sauer references, crystallization parameters were known at the time of filing to readily crystallize proteins with similar crystal structures *as long as the variations introduced did not markedly affect intermolecular crystal contacts or amino acid residues important for protein stability*. The mutation described by Ingram *et al.* had a significant effect in the autoproteolytic activity of TACE, which is correlated with a marked conformational change.

The Office Action cites to Branden *et al.,* Buts *et al.,* Kierzek *et al.*, Wiencek *et al.* and others in support of the allegation that the state of the art for making a protein crystal at the time of the invention was filed was highly unpredictable. Applicants want to clarify the Office's comment that "Applicant concedes that the crystallography art was an unpredictable art at the time of the invention." (Office Action, page 28). Applicants did not concede to this point as alleged by the Office. The position stated in the previous amendment was that the fact that some tedious and time-consuming experimentation may be required to generate crystals of a given protein should not mandate a conclusion that such experimentation is in fact undue, as set forth by the enablement standard set out by

the C.A.F.C. in *Wands,* 858 F.2d 731.

Kierzek *et al. (Biophys Chem* 91:1-20) provides that "each protein crystallizes under a unique set of conditions that cannot be predicted from easily measurable physico-chemical properties" and that "crystallization conditions must be empirically established for each protein to be crystallized." Applicants traverse the Office's position with respect to the relevance of Kierzek *et al.* to the present invention. Applicants had disclosed (and optimized) in the present application several crystallization conditions of the catalytic domain of the TACE polypeptide within the scope of the present claims. The Office is reminded that the claims require the TACE crystals to have the space unit and parameters specified, or the space unit and the TACE amino acid sequence specified. .Not only the TACE polypeptide had been successfully crystallized at the time of filing present application, but also the three dimensional structure of TACE had been resolved. In fact, Applicants disclosed successful crystallization conditions using at least three different buffers and having pH's ranging from 5 to 8.7. Thus, the above-quoted passage by Kierzek *et al.* is simply not relevant to the present application as the successful crystallization of TACE had been performed and conditions for effecting the crystallization are disclosed in the instant application.

To further support the Office's position on the lack of predictability of the art, the Examiner cites Wiencek (1999) *Ann Rev Biomed Eng* 1:505-534 in its teaching that "[p]rotein solubility will change dramatically as pH is altered by ~0.5 pH units... some systems are sensitive to pH changes as small as 0.1 pH units." Applicants respectfully traverse the Office's generalization of the statements in the Wiencek reference. This reference is a general review of crystallization strategies and in the above-quoted passage lists pH as one of several factors that might influence protein solubility during crystallization. The Wiencek reference does necessarily state that all proteins are exceptionally sensitive to pH, but that the effect of pH in protein solubility is protein-dependent. For example, the Wiencek reference provides that:

> The protein of interest will often dictate acceptable pH ranges for crystallization. Only pH values that maintain the folded structure protein are acceptable conditions for protein crystal growth. *(Id* at 514).

The TACE catalytic domain provides an example of a protein that is stable in a wide range of pH values. For example, the specification discloses that TCD formed "crystalline precipitate" over a pH range of 5.0 to 8.7 (a 3.7 change in pH units) *(see e.g.,* paragraphs 103-106 of the specification), thus suggesting that the solubility of TCD is less sensitive to pH variations than other proteins, such as the ones described above in the Wiencek reference. One skilled in the art at the filing date would have recognized that proteins that form crystalline precipitates (such as the TCD), as opposed to amorphous precipitates (which are characteristic of irreversible denaturation), over a wide range of conditions is indicative of a well-folded protein that is more likely to pack productively in a crystal lattice.

Lastly, the Office cites to Branden *et al.* and Buts *et al.* to support the alleged unpredictability of the crystallography art. Buts *et al.* primarily concern mutants of bacterial F17G adhesins having significantly different glycosylation modifications and the effect of such modifications on the crystal structure. Branden reference cited by the Office describes the availability of automated methods for speeding up "the tedious work of reproducibly setting up large numbers of crystallization experiments." *See e.g.,* Branden at page 375.

Applicants submit that the numerous reference cited by the Office in support of the alleged unpredictability of the crystallography art are not relevant to the present claims in view of their scope. Applicants are claiming the ***particular*** TACE crystals that they prepared following the teachings in the specification. The application teaches how to make and use claimed crystals of TACE polypeptides, *e.g.,* crystals of TACE polypeptides of monoclinic space group $P2_1$ and with unit cell dimensions a=61.38 Å, b=126.27 Å, c=81.27 Å, β=107.41°, or crystals of TACE of monoclinic space group $P2_1$ and the reference sequence specified. Applicants had disclosed (and optimized) in the present application several crystallization conditions of the catalytic domain of the TACE polypeptide within the scope of the present claims. In view of the disclosure of the specification and the knowledge in the field of protein crystallography at the filing date, undue experimentation would not be required to make and use the subject matter covered by the claims.

In view of the foregoing, Applicants, therefore, respectfully request reconsideration and withdrawal of the rejections of claims 1-5, 7-9, 11-12, 14-22 and 29-31 under 35 U.S.C. § 112, first paragraph, for failure to satisfy the enablement requirement.

CONCLUSION

In view of the foregoing amendments and remarks, reconsideration is respectfully requested. This application should now be in condition for allowance; a notice to this effect is respectfully requested. If the Examiner believes, after this amendment, that the application is not in condition for allowance, the Examiner is requested to call the Applicant's attorney at the telephone number listed below.

If this response is not considered timely filed and if a request for an extension of time is otherwise absent, Applicant hereby requests any necessary extension of time. If there is a fee occasioned by this response, including an extension fee, that is not covered by an accompanying Deposit Account authorization, please charge any deficiency to Deposit Account No. 50/2762, referencing Attorney Docket No. W2025-702820.

Respectfully submitted,
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